marker. Furthermore, support for the amendments to claim 5 can be found at, *inter alia*, page 12 in the present specification. In addition, claim 16 has been amended to further clarify the claimed invention. Thus, no new matter has been inserted into the application.

Interview Summary

Applicant and Applicant's representative thank Examiners Horlick and Spiegler for the courtesies extended during the interview held on December 4, 2001. It is believed that the interview substantially furthered the prosecution of the application by Dr. Wang explaining some of the nuances of the invention.

Objection to the Specification

The specification has been objected to because at page 9, reference is made to "W," which is not described in the figures. The amended specification does not recite "W" in the specification at the page noted. Therefore, this objection has been overcome.

Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 1-36 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicant traverses this rejection. Reconsideration and withdrawal thereof are respectfully requested.

The Examiner has objected to the recitation of "or optionally" in claim 1 section (d) because the Examiner considers this language to be confusing as to whether the primer extension reagent comprises a terminator nucleotide or absence of a nucleotide that is complementary to the target base at the predetermined position of the nucleic acid.

Applicant notes that the use of the word "optionally" in the claims is permitted under certain circumstances, so long as there is no confusion in the claim language regarding the claimed subject matter. This issue is addressed in the MPEP, Chapter 2173.05(h):

> An alternative format which requires some analysis before concluding whether or not the language is indefinite involves the use of the term "optionally." In *Ex parte Cordova*, 10 USPQ2d 1949 (Bd. Pat. App. & Inter. 1989) the language "containing A, B, and optionally C" was considered acceptable alternative language because there was no ambiguity as to which alternatives are covered by the claim. A similar holding was reached with regard to the term "optionally" in *Ex parte Wu*, 10 USPQ2d 2031 (Bd. Pat. App. & Inter. 1989). In the instance where the list of potential alternatives can vary and ambiguity arises, then it is proper to make a rejection under 35 U.S.C. 112, second paragraph and explain why there is confusion.

In the present situation, the recitation of "one type of terminator nucleotide or optionally, absence of a nucleotide" is not confusing when the invention as a whole as embodied in claim 1 (and now claim 37) is considered. Simply put, in order to practice the claimed invention, the primer extension reaction must be terminated. The means of termination is through the use of a terminator nucleotide such as a dideoxynucleotide, or the absence of the nucleotide that is complementary to the target base. Therefore, given that there is no ambiguity as to which alternative is covered – indeed both are covered – the "optionally" language used in claim 1 is clear and definite. Accordingly, withdrawal of this rejection is respectfully requested.

Claims 16, 18, and 20-22 have been rejected as being indefinite for reciting "allows" in claim 16. Claim 16 has been amended to recite that the moieties are linked to the primer, thereby removing the word "allows" from claim 16. Accordingly, this rejection has been overcome.

Rejection Under 35 U.S.C. § 102(e) Over Soderlund '431 (U.S. Patent No. 6,013,431)

Claims 1-23 and 27-36 have been rejected under 35 U.S.C. § 102(e) as being anticipated over Soderlund '431. Applicant traverses this rejection. Reconsideration and withdrawal thereof are respectfully requested.

The Present Invention

The presently claimed invention provides a rapid and powerful method to detect the presence of a mutation at an indicated target nucleotide on a template nucleic acid. The primer extension reaction mixture is composed of various labeled non-terminator nucleotides that are not complementarily matched to the target nucleotide site. When the template nucleic acid is wild type, the primer extension reaction is terminated by either the addition of a target specific terminator nucleotide such as a dideoxynucleotide, or the absence of the complementary nucleotide. Thus, when there is a mutation at the target nucleotide, this reaction mixture continues to extend the primer beyond the mutated target site so that a plurality of nucleotides are added. Thus, more than one labeled non-terminator nucleotides that are complementary to the template strand are incorporated opposite the mutant base and beyond. In this event, the primer extension reaction proceeds beyond the mutation site until a nucleotide on the template nucleic acid having the same base as the original target nucleotide is encountered, whereupon the primer extension reaction is terminated.

An advantage of the presently claimed invention is that a mutant sample nucleic acid can be detected with a generally greater sensitivity than a conventional method that incorporates only a single labeled nucleotide opposite the mutant site, for example, which results in a newly synthesized counterpart strand that has only one labeled nucleotide incorporated into it. Using

multiple non-terminator nucleotides as in the presently claimed invention results in greater sensitivity of detection of the sample nucleic acid because the signal generated from the newly synthesized strand is multiplied by the addition of the labeled non-terminator nucleotides.

Another advantage of the presently claimed invention over conventionally known methods is that a mutant sample nucleic acid can be detected with a greater level of accuracy than conventional methods that incorporate only a single labeled nucleotide. For instance, when the three types of non-terminator nucleotides are labeled with different types of detectable markers according to the presently claimed invention, only one test needs to be performed to confirm the identity of the mutation by using multiple markers within the same assay.

Soderlund '431

The Soderlund '431 patent generally discloses a method of detecting a mutation at a target site. Soderlund '431 discloses using a single type of labeled nucleotide to detect the target nucleotide by incorporation of a labeled nucleotide that complementarily matches the target site. Soderlund '431 discloses using the one labeled nucleotide to define the target site, such that if the nucleotide (either the terminator or non-terminator) that complementarily matches the target nucleotide is not labeled, the Soderlund '431 method cannot be used to detect any variation in the target nucleotide.

The Examiner's attention is directed to the figures in the Soderlund '431 patent reference. Figure 1(a) shows the addition of a labeled dideoxy terminator nucleotide that is incorporated into the primer extension to accomplish dual purpose of labeling and terminating the primer strand, in which the target site is determined with only a single base terminator.

Figure 1(b) shows adding differentially labeled dideoxy nucleotides to the primer extension reaction to terminate and differentially label the primer strand with only a single base terminator.

Figure 1(c) shows extending the primer with a labeled deoxy nucleotide that is complementary to the target base . According to the scheme set forth in Figure 1 (c), optionally additional unlabeled dideoxy terminator nucleotides corresponding to the nucleotide base immediately after the target base can be included in the reaction mixture to stop the primer extension. Soderlund '431 additionally discloses that the addition of chain terminating ddNTPs provides a means for preventing the incorporation of possibly remaining NTPs (column 8, lines 49-57). As a result, only one labeled nucleotide is incorporated into the primer.

Figure 1(d) shows using more than one differentially labeled dNTP to identify the target site by using nucleotides that are correspondingly matched to the varied target base.

Figure 2 discloses adding labeled terminator nucleotides that are complementary to the target nucleotide, inclusive of circumstances where the 3' end of the primer is a number of nucleotides away from the target site.

Figure 3 discloses using differentially labeled terminator nucleotides that label various target nucleotides.

Considering the Soderlund '431 reference as a whole, the detection method of Soderlund '431 is based on the principle of directly detecting the target nucleotide base by incorporating only one type of a single nucleotide that complementarily matches the target base into the primer. Thus, Soderlund '431 fails to disclose or suggest incorporating multiple types and a plurality of labeled nucleotides to the primer, as the primer extension method disclosed in the Soderlund

'431 reference terminates immediately after incorporation of the single labeled nucleotide complementary to the target nucleotide.

In contrast to the Soderlund '431 patent disclosure, the method of the presently claimed invention is directed to a detection method based on incorporation of multiple types and a plurality of nucleotides into the primer. And furthermore, in contrast to Soderlund '431, none of the nucleotides in the inventive method is complementarily matched to the wild type target site, so that if the target site in the nucleic acid of interest is wild type, there is no primer extension, but if the target site is changed to any other type of nucleotide, the primer will be extended by incorporating multiple types of a plurality of labeled nucleotides. Moreover, the method of the presently claimed invention is directed to the detection of a mutation by the incorporation of multiple nucleotide bases on the extended primer beyond the target base site in the nucleic acid of interest. Finally, if the nucleotide that complementarily matches the target site is not labeled, according to the Soderlund '431 method, no signal would be detected. However, the present invention allows the practitioner to determine the target site by detecting the nucleotides inserted into the primer by continuously extending the primer beyond the target site. Accordingly, Soderlund '431 fails to anticipate the presently claimed invention.

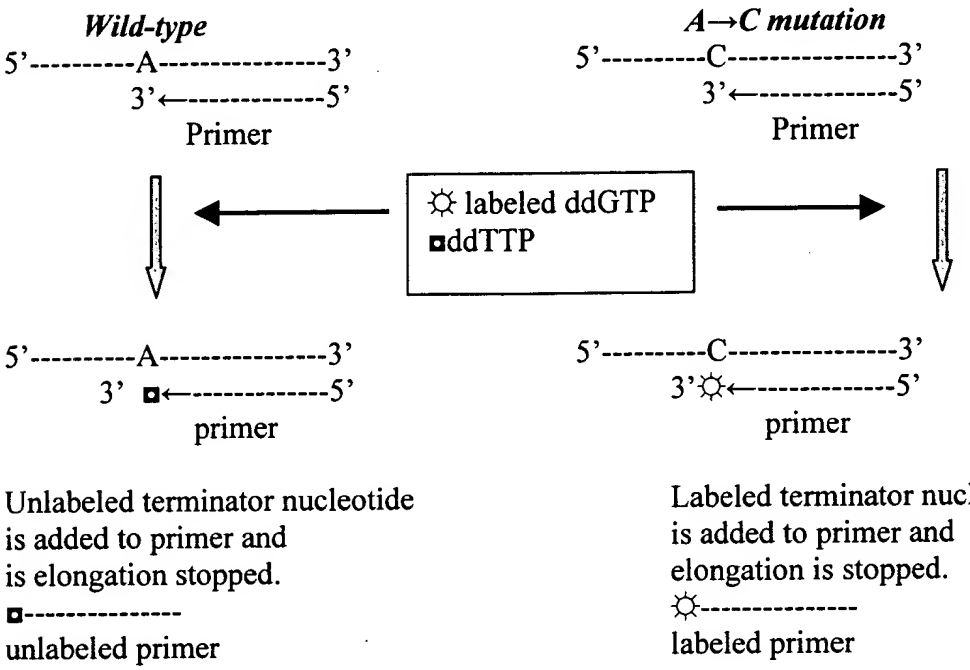
Rejection Under 35 U.S.C. § 102(e) Over Goelet '819 (U.S. Patent No. 5,888,819)

Claims 1-6 and 9-36 have been rejected under 35 U.S.C. § 102(e) as being anticipated by Goelet '819. Applicant traverses this rejection. Reconsideration and withdrawal thereof are respectfully requested.

Goelet '819

Goelet '819 discloses a primer extension reaction mixture comprising a nucleic acid template, an unlabeled dideoxynucleotide that is complementary to the target nucleotide, and a labeled dideoxynucleotide that is not complementary to the target nucleotide. Moreover, Goelet '813 discloses using a single type of labeled nucleotide to detect the target nucleotide by incorporation of only one labeled nucleotide that complementarily matches the target site. If the template is a wild-type version, then the unlabeled dideoxy terminator nucleotide that is complementary to the target nucleotide will be added to the primer, thus terminating the reaction and no labeled primer is produced. However, if the target nucleotide has been mutated from say, "A" to "C," and the labeled dideoxynucleotide that has been added to the mixture is a ddGTP, which is complementary to "C," then the labeled ddGTP will extend the primer, and simultaneously the primer extension will be terminated. This primer extension product emits a signal indicating the presence of a mutation at the target site. To illustrate claim 1 of the Goelet '819 patent, which was noted by the Examiner as being the relevant section of the patent, the Examiner's attention is directed to the summarized scheme as follows:

Goelet '819 method: primer extension using one labeled dideoxynucleotide



Unlabeled terminator nucleotide is added to primer and is elongation stopped.
▫----------
unlabeled primer

Labeled terminator nucleotide is added to primer and elongation is stopped.
☼----------
labeled primer

Goelet '813 fails to disclose or suggest the claimed invention directed to the insertion of a plurality of non-terminator nucleotides as the signal generating component. In contrast to the Goelet '819 patent, the presently claimed invention is directed to a reaction mixture in which non-terminator nucleotides are used as the signal detecting component. Thus, the Goelet '819 patent fails to anticipate the claimed invention.

It is believed that the application is now in condition for allowance. Applicant requests the Examiner to issue a Notice of Allowance in due course. The Examiner is encouraged to contact the undersigned to further the prosecution of the present invention.

The Commissioner is authorized to charge Squire, Sanders & Dempsey's Deposit Account No. **07-1853** for any fees required under 37 CFR §§ 1.16 and 1.17 that are not covered, in whole or in part, by a check enclosed herewith and to credit any overpayment to said Deposit Account No. **07-1853**.

Respectfully submitted,

SQUIRE, SANDERS & DEMPSEY L.L.P.

Dated: January 22, 2002

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VERSION MARKED TO SHOW CHANGES MADE

In the Specification

At page 9, please amend the first paragraph under "Brief Description of the Drawings" as follows:

FIGS. 1A-1C. A schematic drawing of a preferred embodiment of the mutation detection method of the invention is shown. "L" represents the wild-type nucleotide, which can include A, G, C, T, or U. "L*" represents an unlabeled terminator such as a dideoxynucleotide that is complementary to L. "M" represents a mutation at site L, and the mutant nucleotide can include A, G, C, T, or U. [“W” represents a complementary nucleotide to M, and can include A, G, C, T, or U labeled with a detectable marker.] "n" represents one or multiple nucleotides or nucleotide analogues, including A, G, C, T, and U. "y" represents a nucleotide or nucleotide analogue, including A, G, C, T, or U, labeled with a detectable marker and complementary to M or n.

In the Claims

Please amend claims 2-11, 15-16, 23-25, and 27-30:

2. (Amended) The method according to claim [1] 37, wherein the primer is a fragment of deoxyribonucleic or ribonucleic acid, an oligodeoxyribonucleotide, an oligoribonucleotide, or a copolymer of deoxyribonucleic acid and ribonucleic acid.

3. (Amended) The method according to claim [1] 37, wherein the nucleic acid of interest is deoxyribonucleic acid, a ribonucleic acid, or a copolymer of deoxyribonucleic acid and ribonucleic acid.

4. (Amended) The method according to claim [1] 37, wherein the target nucleotide is defined as any known base, which include wild-type or a known mutant base so long as the base is known and it is desired to know its variant.

5. (Amended) The method according to claim [1] 37, wherein the terminator nucleotide is a dideoxyribonucleotide or an analogue thereof and the non-terminator nucleotide is a deoxyribonucleotide or a ribonucleotide or an analogue thereof.

6. (Amended) The method according to claim [1] 37, wherein the terminator nucleotide is unlabeled.

7. (Amended) The method according to claim [1] 37, wherein the terminator nucleotide is labeled with a detectable marker that is different from the marker on the non-terminators.

8. (Amended) The method according to claim [1] 37, wherein in step (d), the duplex from step (c) is contacted with non-terminator nucleotides, wherein each non-terminators is labeled with the same or different detectable marker.

9. (Amended) The method according to claim [1] 37, wherein said detectable marker comprises an enzyme, radioactive isotope, a fluorescent molecule, or a protein ligand.

10. (Amended) The method according to claim [1] 38, wherein said detecting is carried out by mass spectrometry.

11. (Amended) The method according to claim [1] 37, wherein said enzyme is template-dependent.

15. (Amended) The method according to claim [1] 37, wherein the primer comprises one or more moieties that permit affinity separation of the primer from the unincorporated reagent and/or the nucleic acid of interest.

16. (Amended) The method according to claim [1] 37, wherein the primer comprises one or more moieties that [allows linking] links the primer to a solid surface.

23. (Amended) The method according to claim [1] 37, wherein the nucleic acid of interest has been synthesized enzymatically *in vivo*, *in vitro*, or synthesized non-enzymatically.

24. (Amended) The method according to claim [1] 37, wherein the nucleic acid of interest is synthesized by polymerase chain reaction.

25. (Amended) The method according to claim [1] 37, wherein the nucleic acid of interest comprises non-natural nucleotide analogs.

27. (Amended) The method according to claim [1] 37, wherein the sample comprises genomic DNA from an organism, RNA transcripts thereof, or cDNA prepared from RNA transcripts thereof.

28. (Amended) The method according to claim [1] 37, wherein the sample comprises extragenomic DNA from an organism, RNA transcripts thereof, or cDNA prepared from RNA transcripts thereof.

29. (Amended) The method according to claim 27, wherein the organism is a plant, microorganism, bacteria, or virus.

30. (Amended) The method according to claim 28, wherein the organism is a plant, microorganism, bacteria, or virus.

Please add the following new claims 37 and 38:

-- 37. A method for detecting or quantifying a target nucleic acid in a sample by detecting signal from a plurality of labeled nucleotides comprising:

(a) selecting a nucleic acid having a target nucleotide base at a predetermined position in a template of a nucleic acid of interest, wherein the target nucleotide base in original form is not immediately adjacent on its 3' side to an identical base;

(b) preparing an unlabeled primer complementary to a sequence immediately upstream of the target nucleotide base;

(c) treating a sample containing the nucleic acid of interest, if the nucleic acid is double-stranded, so as to obtain unpaired nucleotide bases spanning the specific position, or directly employing step (d) if the nucleic acid of interest is single-stranded;

(d) annealing the primer from (b) with the target nucleic acid from (c) under high stringency conditions to obtain a primer-nucleic acid duplex, wherein the target nucleotide base in the nucleic acid of interest is the first unpaired base immediately downstream of the 3' end of the primer;

(e) mixing the primer-nucleic acid duplex from (d) with a primer extension reaction reagent comprising: (i) three types of non-terminator nucleotides that are not complementarily matched to the target nucleotide, wherein at least one type of the non-terminator nucleotide is labeled with a detectable marker; and optionally (ii) one type of terminator nucleotide that is complementarily matched to the target nucleotide, wherein the terminator nucleotide is not labeled;

(f) performing the primer extension reaction by enzymatic or chemical means, wherein the incorporation of said non-terminator nucleotide and optionally, the terminator nucleotide, to the primer extension depends upon the identity of the unpaired nucleotide base in the nucleic acid template, and wherein when the target nucleotide is changed to any other type of nucleotide, a plurality of non-terminator nucleotides labeled with said detectable marker are sequence-dependently incorporated into the primer extension; and

(g) determining the presence of the mutated nucleotide base at the predetermined position in the nucleic acid of interest by detecting the presence of detectable signal of the non-terminator nucleotides extended from the primer. --

-- 38. A method for detecting or quantifying a target nucleic acid in a sample comprising:

(a) selecting a nucleic acid having a target nucleotide base at a predetermined position in a template of a nucleic acid of interest, wherein the target nucleotide base in original form is not immediately adjacent on its 3' side to an identical base;

(b) preparing a primer complementary to a sequence immediately upstream of the target nucleotide base;

(c) treating a sample containing the nucleic acid of interest, if the nucleic acid is double-stranded, so as to obtain unpaired nucleotide bases spanning the specific position, or directly employing step (d) if the nucleic acid of interest is single-stranded;

(d) annealing the primer from (b) with the target nucleic acid from (c) under high stringency conditions to obtain a primer-nucleic acid duplex, wherein the target nucleotide base in the nucleic acid of interest is the first unpaired base immediately downstream of the 3' end of the primer;

(e) mixing the primer-nucleic acid duplex from (d) with a primer extension reaction reagent comprising three types of non-terminator nucleotides that are not complementarily matched to the target nucleotide;

(f) performing the primer extension reaction by enzymatic or chemical means, wherein the incorporation of said non-terminator nucleotide into the primer extension depends upon the identity of the unpaired nucleotide base in the nucleic acid template, wherein when the target nucleotide base is changed to another type of nucleotide, a plurality of non-terminator nucleotides are sequence-dependently incorporated into the primer extension; and

(g) determining the presence of the mutated nucleotide base at the predetermined position in the nucleic acid of interest by detecting the length of the primer extended strand, wherein if the primer extended strand is longer than the primer the presence of the mutated nucleotide base is indicated. --